(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 November 2001 (29.11.2001)

PCT

(10) International Publication Number
WO 01/90297 A1

(51) International Patent Classification[7]: C12M 1/30, A61B 10/00

(21) International Application Number: PCT/FI01/00490

(22) International Filing Date: 21 May 2001 (21.05.2001)

(25) Filing Language: Finnish

(26) Publication Language: English

(30) Priority Data:
20001254 25 May 2000 (25.05.2000) FI

(71) Applicant *(for all designated States except US)*: **CELLOMEDA OY** [FI/FI]; BioCity, Tykistökatu 6 A, FIN-20520 Turku (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HUOVINEN, Pentti** [FI/FI]; Nikkarmäenkuja 1, FIN-20380 Turku (FI). **VILJANTO, Jouko** [FI/FI]; Terhokatu 18, FIN-20720 Turku (FI). **LÖNNQVIST, Kurt** [FI/FI]; Nylandsgatan 12 B 30, FIN-20500 Åbo (FI). **MERIKALLIO, Hannu** [FI/FI]; Pulmussuontie 13, FIN-20300 Turku (FI).

(74) Agent: **TURUN PATENTTITOIMISTO OY**; P.O. Box 99, FIN-20521 Turku (FI).

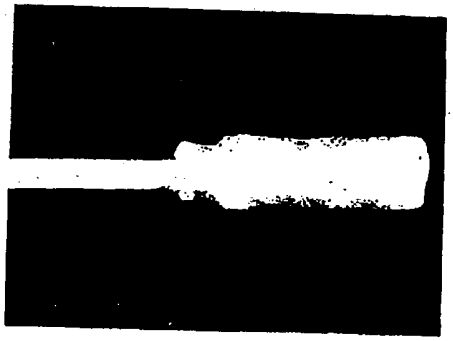
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: DEVICE INTENDED FOR SAMPLE COLLECTION IN DIAGNOSTICS OF DISEASES AND ITS USE

a



b

(57) Abstract: The invention relates to a device intended for sample collection in the diagnostics of diseases, the device comprising a stick (1) and on the outer surface of its tip a porous part (2) which absorbs, preserves and yields fluid and any cells, microbes and the like possibly present therein, which part is according to the invention a cellulose sponge, preferably a pressure molded cellulose sponge. The invention also relates to the use of the above-mentioned sample collection device for collecting a sample from an infected area containing *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* bacteria.

WO 01/90297 A1

patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Device intended for sample collection in diagnostics of diseases and its use

The present invention relates to a device intended for sample collection in diagnostics of diseases, and in particular to a device which comprises a stick and on the outer surface of its tip a porous part which absorbs, preserves and yields fluid and any cells, microbes and the like possibly contained in the fluid. The invention also relates to the use of the above-mentioned device.

Correct diagnosis is the cornerstone in the treatment of diseases, such as infectious diseases. Correct diagnosis, in turn, requires that a sample as representative as possible is obtained from the target of examination, such as an infected area, and therefore it is essential in terms of diagnosis to obtain a sufficient quantity of sample for testing. It is also important that the device used for sample collection is economical and simple to use, since these factors lower the threshold of sample collection and lower the costs of diagnostics. In terms of diagnostics it is not sufficient that a representative sample is obtained from the target of examination; it is necessary to preserve this sample in the sample collection device until the time of testing, and it is necessary for the sample in the sample collection device to be capable of being transferred, for further investigation, for example, to a test tube or a Petri dish in such a manner that the obtained sample represents the target of examination as precisely as possible.

There are previously known sample collection swabs developed for the above-mentioned purposes, wherein there is a wad at the tip of a stick, for example a wooden stick, which wad may be of cotton, a cotton-based material or artificial fiber. However, these materials are not especially absorbent, they do not very well absorb cells and microbes present in a fluid, it is difficult to determine from the wad whether it has in general absorbed any sample, since the wad does not swell upon meeting a fluid, the material at the tip of the stick does not especially well keep alive any cells and microbes which have possibly passed into it in a fluid, and the transfer of the sample to the point of testing may remain deficient. Such prior-known sample collection swabs thus do not in the best manner fulfill the basic prerequisites of diagnostics with respect to as representative a sample collection as possible, good preservation of sensitive cells and bacteria, and as complete a transfer as possible for diagnosis of the recovered sample. Instead, these prior-known sample collection swabs are indeed inexpensive, but, for the above-mentioned reasons, their performance is not the best possible, and it is difficult to determine from

them whether any sample collection has even occurred. This is a problem in particular when a sample is taken from body cavities, from which it cannot be seen whether there is in the sample collection area a sufficient amount of sample material for sample collection.

The object of the present invention is to eliminate the above disadvantages and to provide a device intended for sample collection in the diagnostics of diseases, the device comprising a stick and, on the outer surface of its tip, a porous part which absorbs, preserves and yields fluid and any cells, microbes and the like possibly present in the fluid. According to the invention the said porous part is a cellulose sponge.

The use of a cellulose sponge in sample collection is previously known. However, the cellulose sponge has been fitted inside the sample collection device, in which case the fluid to be tested is sucked or absorbed into the sample collection device, which may be, for example, a micropipette or an injection syringe (Copeland *et al.*, Invest Ophthalmol Vis Sci, 1982 Jan 22:1, 103-10, and US patent publication 3,704,206) or a flexible capillary tube inserted into an operation wound (FI patent publication 77,569). However, such sample collection devices are not suited for sample collection from targets for which sample collection swabs have been designed.

The cellulose sponge on the outer surface of the tip of the sample collection swab according to the present invention is preferably one which contains intercommunicating micro- and macropores, the micropores being as large as possible, the median of the micropore distribution being preferably 5 – 15 µm, and the macropores are as large as possible, the median of their distribution being preferably 0.4 – 0.9 mm. Such a cellulose sponge absorbs well a fluid and any cells, microbes and the like carried therein, preserving them for long periods for the diagnosis of the sample.

The manufacture of cellulose sponge is prior known, and it has been described, for example, in FI patent publication 77,569.

The cellulose sponge on the outer surface of the tip of the sample collection swab according to the invention is especially preferably of pressure molded cellulose sponge having the advantage that, upon wetting, it swells vigorously, efficiently absorbing fluid and, along with the fluid, cells and microbes. Since the cellulose sponge is on the outer surface of the swab, the sample collector is able at the same time to assess visually from the swelling of the cellulose sponge whether the sample

collection has succeeded, and even a small amount of fluid can be collected with precision. The outer diameter of the compression molded cellulose sponge at the tip of the swab according to the invention is in the dry state, for example, approximately 1 – 10 mm and, when completely swollen, up to approximately 2 – 30 mm. Owing to its small outer diameter, the swab fits into even narrow cavities, with room to swell in them, and owing to strong change of diameter, i.e. swelling, of the sponge, the success of sample collection can easily be verified.

The length of the stick-like part of the sample collection device according to the invention is preferably many times greater than the length of the cellulose sponge at its tip, and the stick-like part is preferably of a flexible material, such as wood, plastic or metal. A long wooden stick has the advantage that it can be easily severed, for example, by pressing it against the inner wall of the test tube serving as the recipient of the sample, whereupon the cellulose sponge and only that part of the wooden stick which has not been touched by hand remain in the test tube. In other respects the flexibility properties of the stick are selected so that the cellulose sponge at its tip can be pressed with sufficient force against the sample collection surface.

The present invention additionally relates to the use of the above-mentioned sample collection swab for collecting a sample from an infected area with sensitive microbes, such as *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* bacteria. It has been observed that such sensitive bacteria retain their proliferation capacity for even long periods in the cellulose sponge at the tip of the swab according to the invention, which is substantially important in terms of correct diagnosis.

In its preferred embodiment the invention thus relates to a sample collection swab which has on the outer surface of its tip a pressure molded sponge having a predetermined pore structure, and which is used for sample collection in the diagnostics of diseases. Good absorption of the sample into the cellulose sponge swab can be seen as an increase in the volume of the cellulose sponge. The cellulose sponge absorbs and yields a significantly larger amount of sample material than do previously used sample collection swabs. Furthermore, its ability to preserve, for example, sensitive bacteria is better than that of prior sample collection swabs made of other materials.

The cornerstone in the treatment of infectious diseases is thus correct diagnosis, as noted above. In order to ensure correct diagnosis, a sample is taken from the person examined in order to identify the causative agent of the disease. Although methods having different sensitivities are used in the identification, the amount of the causa-

tive agent in the sample tested is essential in the methods. Therefore it is important to obtain as large a sample as possible for further investigations. When using the present-day sample collection devices the sample taker does not always know whether the sample is sufficiently representative, since it is not possible to estimate visually the absorbed quantity of the sample, especially a small quantity.

Since the costs of the microbiologic testing of a sample and the collection of the sample are considerably higher (> 100-fold) than those of the sample collection swab, improving the quality of sample collection increases the cost-efficiency of the entire process.

The invention is described below in greater detail with reference to the accompanying drawings, wherein Figure 1a depicts the sample collection swab according to the invention before sample collection and Figure 1b depicts the sample collection swab according to the invention after being dipped in water, whereas Figures 2 and 3 depict bar diagrams of the amounts of broth culture medium (BHI) and human blood absorbed and yielded by the sample collection swab according to the invention and a few sample collection swabs known *per se*.

In Figures 1a and 1b the elongated stick, made of plastic, metal or wood, of the sample collection swab according to the invention is indicated by reference numeral 1 and the pressure molded cellulose sponge part attached around its tip by reference numeral 2. A comparison between Figures 1a and 1b shows that the diameter of the completely swollen cellulose sponge part 2 of the sample collection swab shown in Figure 1b is significantly larger than in Figure 1a, which indicates that the sample collection has been successful. The diameter of even a completely swollen cellulose sponge part 2 is not substantially larger than that of previously known sample collection swabs, which is significant in terms of the success of sample collection, for example, from a narrow nasopharynx or auditory canal.

In Figures 2 and 3, the capacity of the sample collection swab according to the invention to absorb and yield various fluids, measured in grams, is compared with that of known and widely used sample collection swabs: Dacron®, Sterilin® and Transpocult®. The wad parts of these known sample collection swabs do not swell upon being wetted, and the wad of the last-mentioned is of cotton, and the wads of the other two are of artificial fiber (Dacron®).

A comparison of a sample collection swab according to the invention, comprising a pressure molded cellulose sponge, with the above-mentioned known sample collec-

tion swabs showed that it absorbs, measured in weight units (g), 1.3-1.9 times more and yields 3.5-4.6 times more sample material than do the control swabs. The measurements were made before the swabs were dipped in the sample material, after the absorption of the sample material, and after the sample swab had been rotated on the surface of an agar plate without breaking the agar surface. In the study, the sample material used was a broth medium (BHI: brain heart infusion broth) in the diagram of Figure 2 and human blood in the diagram of Figure 3.

The survival, after sample collection, of sensitive bacteria, *Streptococcus pneumoniae* and *Neisseria gonorrhoae*, in the cellulose sponge swabs according to the invention and in the above-mentioned control swabs was studied at different temperatures and media.

Example 1

Conditions: *Streptococcus pneumoniae* bacterium; BHI medium (brain/heart infusion broth); room temperature; 4 different initial concentrations of the bacterial suspension within the range 7.75x 10E5 CFU/ml – 12x 10E6 CFU/ml; after the absorption of the sample the swab was stored in a sterile closed glass tube:
The counts of bacterial colonies, cultured on a blood agar plate, remained unchanged in the cellulose sponge swab for 96 hours. In the Dacron® swab and the Sterilin® swab the colony counts decreased already after 6 hours, and after 96 hours the bacteria were dead at all concentrations. With the Transpocult® swab, growth was observed at 96 hours at only two concentrations.

Example 2

Conditions: *Streptococcus pneumoniae* bacterium; BHI medium; temperature +4 °C; 3 initial concentrations of the bacterial suspension within the range 12x 10E6 CFU/ml – 29x 10E6 CFU/ml; after the absorption the swab was stored in a sterile closed glass tube:
The counts of bacterial colonies, cultured on a blood agar plate, remained unchanged in the cellulose sponge swab for 96 hours. In the control swabs the colony counts decreased already at 24 hours (Dacron® and Sterilin®) and at 96 hours the colony counts had dropped 5-10 times lower at the two bacterial concentrations used than in the cellulose sponge swab.

Example 3

Conditions: *Neisseria gonorrheae* bacterium; BHI medium; room temperature; 5 different initial concentrations of the bacterial suspension within the range 1.7x 10E4 CFU/ml – 6.2x 10E5 CFU/ml; after the absorption of the sample the swab was stored in a sterile closed glass tube:

The counts of colonies, cultured on a gonococcus plate, remained unchanged for 6 hours in the cellulose sponge swab, at four bacterial concentrations, and at 24 hours the colony counts were less than 10 % of the initial colony count at all the bacterial concentrations. With the Sterilin® swab the results were similar to those with the cellulose sponge swab. By contract, in the Dacron® swab and the Transpocult® swab the colony counts dropped dramatically already at 4 hours, and the bacteria had died at 24 hours at all the concentrations.

Example 4

Conditions: *Neisseria gonorrhoeae* bacterium; BHI medium; temperature +4 °C; 2 different initial concentrations of the bacterial suspension within the range 1.7x 10E4 CFU/ml – 6.2x 10E5 CFU/ml; after the absorption of the sample the swab was stored in a sterile closed glass tube:

In the cellulose sponge swab the bacterium could be cultured on a gonococcus plate at both bacterial concentrations at 24 hours and at one of the concentrations still at 48 hours. In the control sticks the culture was negative at both concentrations at 24 hours.

Example 5

Conditions: *Neisseria gonorroeae* bacterium, two culture media, modified Stuart and Amies charcoal; temperature +4 °C; 4 different initial concentrations of the bacterial suspension within the range 4.2x 10E4 CFU/ml – 5.0x 10E4 CFU/ml.

At two bacterial concentrations the bacterium could be cultured from the cellulose sponge swab at 48 hours in the Amies charcoal transport medium and at all bacterial concentrations at 48 hours in the Stuart medium. From the Sterilin® swab the bacterium could no longer be cultured in the Amies charcoal culture medium at 24 hours, the Transpocult® swab in the Stuart medium gave a positive result at three bacterial concentrations at 24 hours, but at 48 hours the cultures were negative.

Claims

1. A device intended for sample collection in the diagnostics of diseases, the device comprising a stick (1) and, on the outer surface of its tip, a porous part (2) that absorbs, preserves and yields fluid and any cells, microbes and/or the like possibly present therein, **characterized** in that the porous part (2) is a cellulose sponge containing intercommunicating micro- and macropores.

2. The device according to Claim 1, **characterized** in that the distribution median of the micropores is 5 – 15 µm and that of the macropores 0.4 – 0.9 mm.

3. The device according to Claim 1 or 2, **characterized** in that the cellulose sponge part (2) is of cellulose sponge that has been pressure molded at the tip of the stick (1) and swells vigorously when wetted.

4. The device according to Claim 3, **characterized** in that the outer diameter of the pressure molded cellulose sponge part (2) at the tip of the stick (1) is approximately 1 – 10 mm when dry and approximately 2 – 30 mm when fully swollen.

5. The device according to any of the preceding claims, **characterized** in that the stick (1) is many times longer than the porous cellulose sponge part (2) which is at its tip and absorbs, preserves and yields fluid and any cells, microbes and the like possibly present in the fluid, and that the stick (1) is of a resilient material, preferably wood, plastic or metal.

6. The use of a device intended for sample collection in the diagnostics of diseases, the device comprising a stick (1) and on the outer surface of its tip a cellulose sponge part (2) which absorbs, preserves and yields a fluid-containing sample and contains intercommunicating micro- and macropores, **characterized** in that it is used for collecting a sample from an infected area which contains sensitive microbes, such as *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* bacteria.

Figure 1a

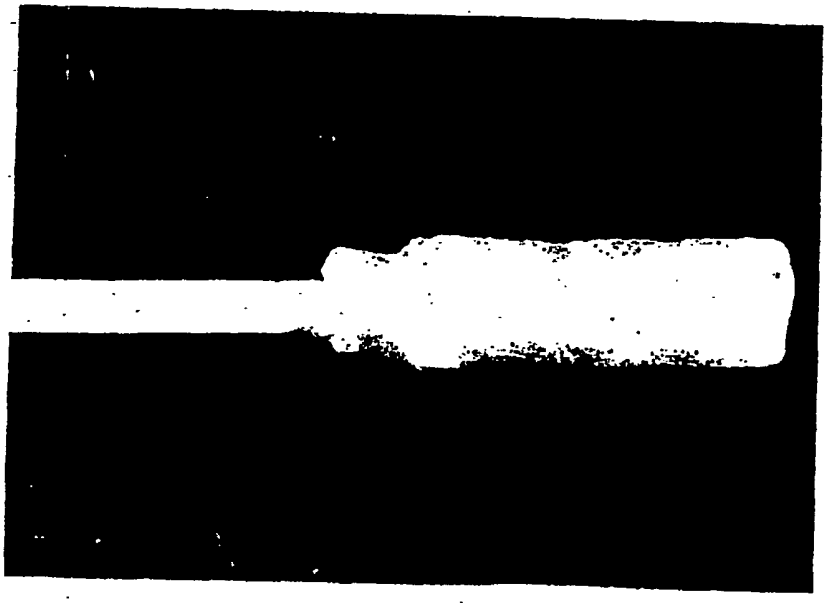


Figure 1b

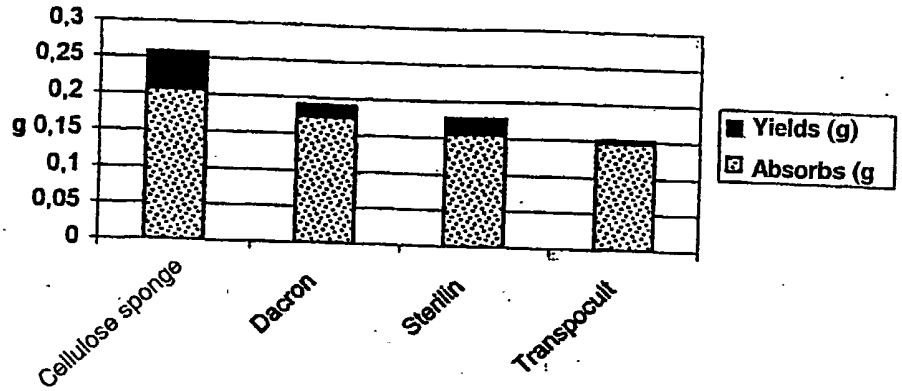


Figure 2. BHI broth (N = 82)

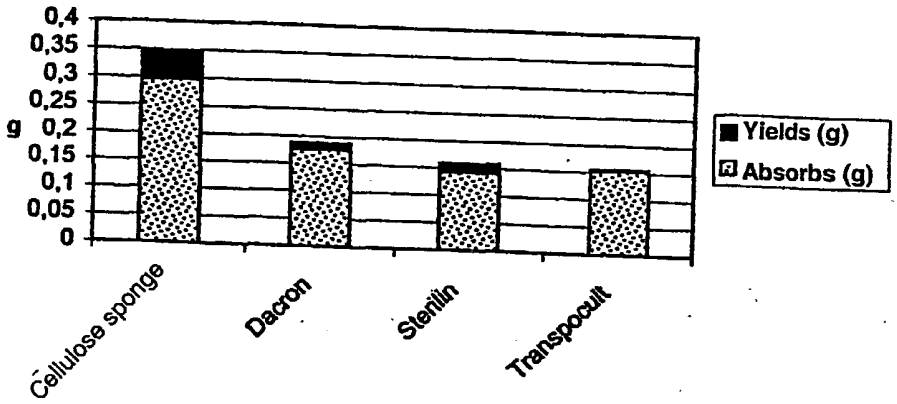


Figure 3. Blood (N = 141)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00490

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12M 1/30, A61B 10/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12M, A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4749655 A (MONTHONY ET AL), 7 June 1988 (07.06.88), column 2, line 45 - line 52	1,6
Y	column 4, line 61 - line 63	2-5
	--	
X	WO 9525948 A1 (CELSIS INTERNATIONAL PLC), 28 Sept 1995 (28.09.95), page 7, line 31 - line 35	1
Y		2-5
	--	
X	US 4953560 A (SAMUELS), 4 Sept 1990 (04.09.90), abstract	1
Y		2-5
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2001	15 -10- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00490

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9747764 A1 (VALTION TEKNILLINEN TUTKIMUSKESKUS), 18 December 1997 (18.12.97), page 8, line 7 - line 10; page 4, line 21 - page 5, line 18	1,6
Y	--	2-6
Y	US 5113871 A (VILJANTO ET AL), 19 May 1992 (19.05.92), figure 2, abstract	2-5
Y	--	2-5
A	US 3704206 A (RONALD FREAKE ET AL), 28 November 1972 (28.11.72), column 5, line 52	1-6
	--	
	--	

Form PCT/ISA/210 (continuation of second sheet) (July 1998).

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/01

International application No.
PCT/FI 01/00490

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4749655 A	07/06/88	NONE	
WO 9525948 A1	28/09/95	AU 1955595 A	09/10/95
		GB 9405590 D	00/00/00
US 4953560 A	04/09/90	NONE	
WO 9747764 A1	18/12/97	FI 101809 B	00/00/00
		FI 962408 A	12/12/97
US 5113871 A	19/05/92	AT 107847 T	15/07/94
		AU 618016 B	12/12/91
		AU 2088688 A	13/02/89
		CA 1325971 A	11/01/94
		DE 3850504 D,T	13/10/94
		DK 5990 A	09/03/90
		DK 164984 B,C	28/09/92
		EP 0395642 A,B	07/11/90
		SE 0395642 T3	
		FI 77569 B,C	30/12/88
		FI 873075 D	00/00/00
		HU 52933 A	28/09/90
		HU 203276 B	29/07/91
		JP 2811639 B	15/10/98
		JP 3500610 T	14/02/91
		NO 900180 A	12/01/90
		NZ 225366 A	27/11/90
		SU 1797484 A	23/02/93
		WO 8900403 A	26/01/89
US 3704206 A	28/11/72	NONE	
US 4136680 A	30/01/79	CH 636443 A	31/05/83
		DE 2724522 A	22/12/77
		FR 2353640 A,B	30/12/77
		GB 1587174 A	01/04/81
		IT 1077218 B	04/05/85
		JP 52153490 A	20/12/77